PUERTO RICO EXPERIMENT STATION
of the
UNITED STATES DEPARTMENT OF AGRICULTURE
MAYAGUEZ, PUERTO RICO

BULLETIN NO. 40

THE BIOLOGY OF METAGONISTYLUM MINENSE TNS. A PARASITE OF THE SUGARCANE BORER

By
KENNETH A. BARTLETT
Entomologist

Issued December 1941

UNITED STATES DEPARTMENT OF AGRICULTURE
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CONTENTS

INTRODUCTION

Metagonistylum minense Tns. is a dipterous larval parasite of the sugarcane borer (*Diatraea saccharalis* (F.)). It was first described in 1926 by C. H. T. Townsend (*17*)[1] from a single specimen collected in the State of Minas Geraes, Brazil, but at the time nothing was known of its host relationships. Townsend (*18*, *pp. 341–343*) places the genus *Metagonistylum* in the superfamily Oestroidea: family Tachinidae; subfamily Germariinae; tribe Germariini.

Since the initial introduction by Myers (*14*) into British Guiana in 1932, it has been distributed to many of the important sugar-growing areas of the Western Hemisphere. In 1937 Harland (*9*) published an account of what he considered to be a biological race of *M. minense* existing in the State of São Paulo, Brazil. In 1939 Bartlett (*3*) published an account of the collection of this biological race in São Paulo and of its subsequent introduction into Puerto Rico. Since then this strain has been introduced into Barbados, British West Indies, by Tucker (*19*) and into the southern United States (*10*).

DISTRIBUTION AND HOST RELATIONSHIPS

The native habitat of *Metagonistylum minense* is probably limited to the South American continent, in a region bounded, roughly, on

[1] Italic numbers in parentheses refer by authors to Literature Cited, p. 18.

the north by the Equator, on the south by a line passing through the State of São Paulo, Brazil, and the Bolivian Chaco, its east and west boundaries being the Atlantic Ocean and the east side of the Andes. Within these limits, as suggested by Harland (*9*) there may be more than one biological race of the same species.

The host relationship of this parasite was first recorded in 1933 by Monte (*12*) who stated that he had reared *M. minense* from *Diatraea saccharalis* (F.) larvae that had been collected in the State of Minas Geraes, Brazil, in 1931. Myers was the first to recognize the possibilities of introducing the fly into other areas where the sugarcane borer was present. His discovery of the fly as a parasite of *D. saccharalis* infesting water grasses in the Amazon was made independently of the observations by Monte.

According to Myers (*14*) the favorite host of *M. minense* in the Amazon region is *D. saccharalis*, but he also found it parasitizing another species, *Diatraea albicrinella* Box. Cleare (*6*) in British Guiana and Box (*4*) in St. Lucia, British West Indies, have shown that *M. minense* also attacks another sugarcane borer (*Diatraea canella* Hampson) but that the percentage of parasitization in the field is negligible. An attempt by Cleare (*5*) to rear a race of *M. minense* adapted to *D. canella* was unsuccessful. Only one of these species, *D. saccharalis*, occurs in Puerto Rico, and all attempts to rear *M. minense* on related lepidopterous species have failed.

Since its first introduction into British Guiana this parasite has been colonized in St. Lucia (*4*), Puerto Rico (*2*, *8*), Trinidad (*7*), Cuba, and Barbados (*19*) in the West Indies, Louisiana, Florida (*2*), and, according to unpublished information available to the author, in Guadeloupe.

The Amazon strain of *M. minense* apparently requires very wet and humid conditions for survival. Collections of *Diatraea* borers by Myers in the Amazon region were made largely from beds of floating grasses. In British Guiana a large part of the sugarcane areas are below sea level, and the canefields are usually completely surrounded by water.

In some cane-growing sections of St. Lucia the annual precipitation may exceed 150 inches, and even the drier coastal areas receive close to 80 inches.

The São Paulo strain of *M. minense* may be better adapted to sugar-growing areas receiving less rainfall, such as those of Puerto Rico, Cuba, and the continental United States. At Piracicaba in the State of São Paulo, Brazil, the average mean rainfall over a period of 10 years was 51.02 inches with the wet and dry seasons clearly marked.[2]

ECONOMIC IMPORTANCE

According to Cleare (*7*), the cost of originally introducing *Metagonistylum minense* into British Guinia from the Amazon, exclusive of colonization by the sugar estates, did not exceed $24,000. In 1937 Cleare (*6*) stated that the fly was parasitizing an average of 13.7 percent of *Diatraea saccharalis* in British Guiana, as compared with the condition found by Myers (*13*) in 1931, prior to its introduction,

[2] Information obtained from records at Instituto Agronômico do Estado de São Paulo, Campinas, Brazil.

when he stated that the combined parasitism of eight other important parasites of this borer was only 6.9 percent. Cleare concludes that "there can be no doubt as to the value of the introduction, nor that the cost of the undertaking has been amply repaid."

According to Myers (*14*), the parasitization by this fly in its native habitat varied tremendously from place to place, his collections in the Amazon region ranging from 10 to 40 percent.

The Amazon fly rapidly became established in St. Lucia after its introduction from British Guiana in November 1934. According to Box (*4*) this occurred with a rapidity almost without parallel in the history of biological control. In 1938, after completing a survey of the Amazon fly in St. Lucia, Box stated the infestation of canes by moth borers to be about half of that prevailing before the introduction of the parasite.

In São Paulo, Brazil, Harland (*9*) reported a parasitism of approximately 56 percent in 1937. In 1939 Bartlett (*3*) made collections at Piracicaba and Campinas in the State of São Paulo and found a parasitization of 33.9 percent and 39.3 percent, respectively, in the two localities.

DESCRIPTIONS

ADULT

The adult of *Metagonistylum minense* (fig. 1) is readily distinguishable from any of the other known dipterous parasites of *Diatraea saccharalis*. The black, prominent antennae, which are usually held in an extended position, are a unique characteristic of the species. The flies vary considerably in size, depending upon the number reared from a single host and also the size of the host larva. The average fly measures 8.0 mm. in length, extremes found being 5.25 and 10.50 mm. long.

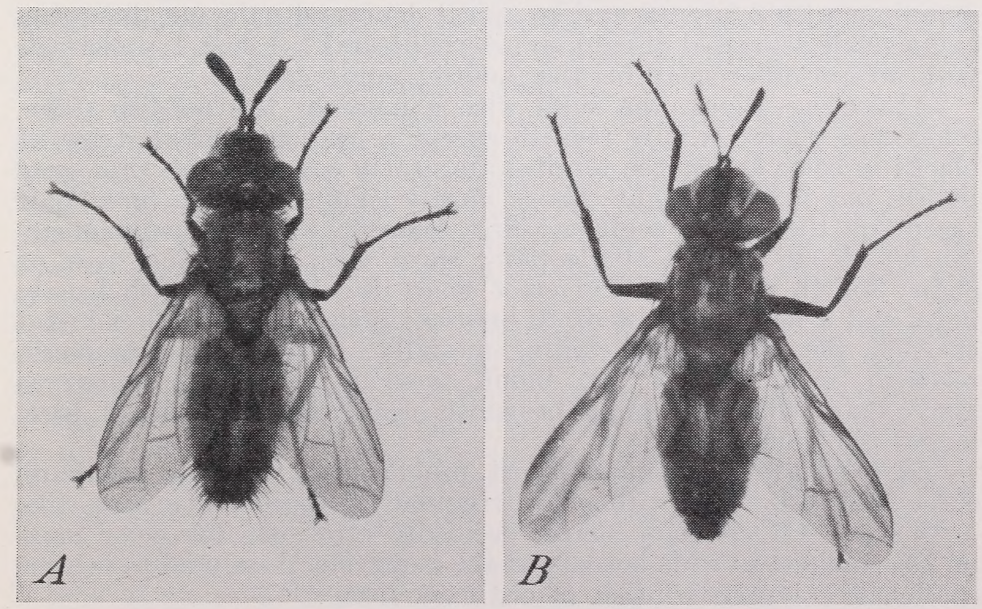

FIGURE 1.—*A*, Adult male of *Metagonistylum minense; B*, adult female of *Metagonistylum minense.*

The female is readily distinguished from the male by the shape of the abdomen, which in the latter is distinctly blunt, and also by the presence of heavier bristles at the tip of the abdomen of the male. The abdomen of the female is heart-shaped, gradually curving to a point.

The following is the original description of the species by Townsend (*17*):

"Cco 9; caz 7. 1 0, Vicosa, Minas Geraes, 22 de Abril, em folhagem.

"Cb prateada, pfrlia preto-polidas, vistas de cima; frlia pardo-claras; ant pardas; plp fulvos; thx, scutello e abd preto-polidos, levement prateados, o abd obscurament roxeado, especialmente nos lados do 2seg; pernas pretas; azas enfumacadas nas costas e nas nervuras; sq brancas."

The species was subsequently redescribed by Aldrich (*1*) from 3 females and 2 males reared at Santarem, Amazon, Brazil, by J. G. Myers, as follows: "Black including antennae and legs; scutellum more or less reddish.

"*Male—Head.*—Vertex 0.31 of head width, the frons widening considerably; parafrontals and parafacials with smooth silvery pollen becoming thin near vertex so that the upper parafrontals appear shining; outer vertical about one-half the inner; a distinct pair of erect parallel post-ocellar bristles; back of head slightly swollen, with broad silvery orbits, a few of the hairs below pale; palpi of ordinary size, blackish except extreme tips, which are distinctly yellow below.

"*Thorax.*—Subshining black with cinereous or rather glaucous pollen, leaving two pairs of shining black stripes, the inner narrow, the outer becoming narrow at the suture and blending behind into a single large subshining area, only visible in posterior view. Chastotaxy: acrostichal 3, 3 (all rather small); dorsocental 3, 4 (rather small except hindmost); humeral 4; posthumeral 2; presutural 1; notopleural 2; intraalar 2 (far back, rather hairlike); postalar 2; scutellum with 3 laterals and an apical depressed pair, not diverging and a small depressed discal pair; sternopleural 1, 1 or 2, 1, sometimes with several other small bristles; ptero-pleural small.

"*Abdomen.*—Black, the sides with a more or less reddish tinge, sometimes almost wholly reddish except a median ventral stripe of black; in some angles the tergites are nearly covered with thin gray pollen, but in other light this is mostly confined to the basal half except on the fourth segment; first segment without median marginals; second with a single pair; third with a marginal row of eight; fourth with a row of erect small discals mixed with bristly hairs and a marginal row of 10 bristles; fifth sternite with a broad excision, the sides short, shining mesially; genital segments small, blackish, inner forceps flat, blunt, tapering, hardly separated to apex; outer forceps shining brownish-black, slender, blunt, a little longer than inner.

"*Legs.*—Claws and pulvilli short, middle tibia with one bristle on outer front side; hind with a few irregular bristles on outer hind side.

"*Wings.*—Of ordinary shape, decidedly brownish, the color more or less confined to wide borders of the veins, but these tend to become confluent; calypters whitish, of ordinary form.

"*Female.*—Frons 0.37 of head width, the parafrontals shining black to a larger extent than in the male; two pairs orbital bristles which

are both proclinate in two of the specimens, but in the third the upper one is unmistakably reclinate; abdomen somewhat keeled below and with numerous depressed hairs along the middle; no visible ovipositor."

The strains of *Metagonistylum minense* vary in the amount of reddish or yellowish coloring on the abdomen. In general such variations occur in definite patterns according to different localities. There is also a color variation in the wings from gray to light brown.

Specimens from the São Paulo, Brazil, area are nearly always black throughout, but specimens with a slight reddish coloring on the abdomen do occur there and the progeny resulting from matings of these reddish specimens in the laboratory is predominantly the pure black. The wings of the São Paulo strain are light gray in color.

The Amazon material always has some reddish or yellowish coloration on the abdomen but the amount varies. The two strains mate readily, and the progeny are a mixture of pure blacks and color variants.

Tucker (*19*) states that the crossings of the two strains result in intermediate colorations which can be distinguished by trained observers. He also states that "fecundity appears greater in the wet-area strain than in the dry-area according to the number of maggots available for inoculation on dissection of the flies." As suggested by him, and confirmed by our observations, it would appear that this is not a difference in fecundity but results from the fact that the Amazon or wet-area strain matures 24 to 48 hours earlier than the São Paulo or dry-area strain.

OVISAC AND EGG

The potential reproductive capacity of *Metagonistylum minense* is high, but without doubt many of the eggs are not deposited before the natural death of the individual. In an unfertilized female the ovisac remains undeveloped and shapeless. After mating and completion of the gestation period the ovisac has the appearance of a spiral tube with the posterior end narrowing to a diameter which permits only single eggs to lie in a longitudinal position.

The number of eggs contained in the ovisac of a female varies greatly, being found to be from 330 to 714. The size of the individual is an important factor in potential reproductive capacity. Large females contained 200 to 300 more eggs than smaller ones. Cleare (*7*) has shown after extensive observations that the duration of mating does not appear to have any effect on the number of larvae and ova produced by a female.

In no case under laboratory conditions has a female fly survived for a sufficient length of time for all of the eggs in the ovary to mature, and it seems questionable if this ever occurs under field conditions.

The size and shape of the egg (fig. 2, *A*) may be determined only by dissection, as the female deposits eggs which hatch immediately. Both the mature and undeveloped eggs are similar in shape and average 0.55 mm. long by 0.15 mm. wide at the midsection.

LARVA

Immediately after hatching, the first-instar larva (fig. 3, *A*) measures from 0.60 to 0.70 mm. in length. The body is semitransparent and divided into 10 segments, the margins of which bear short

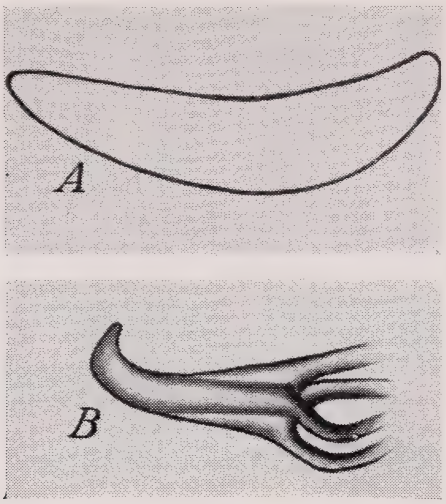

FIGURE 2.—*A*, Egg of *Metagonistylum minense; B*, mandibles of first-instar larva.

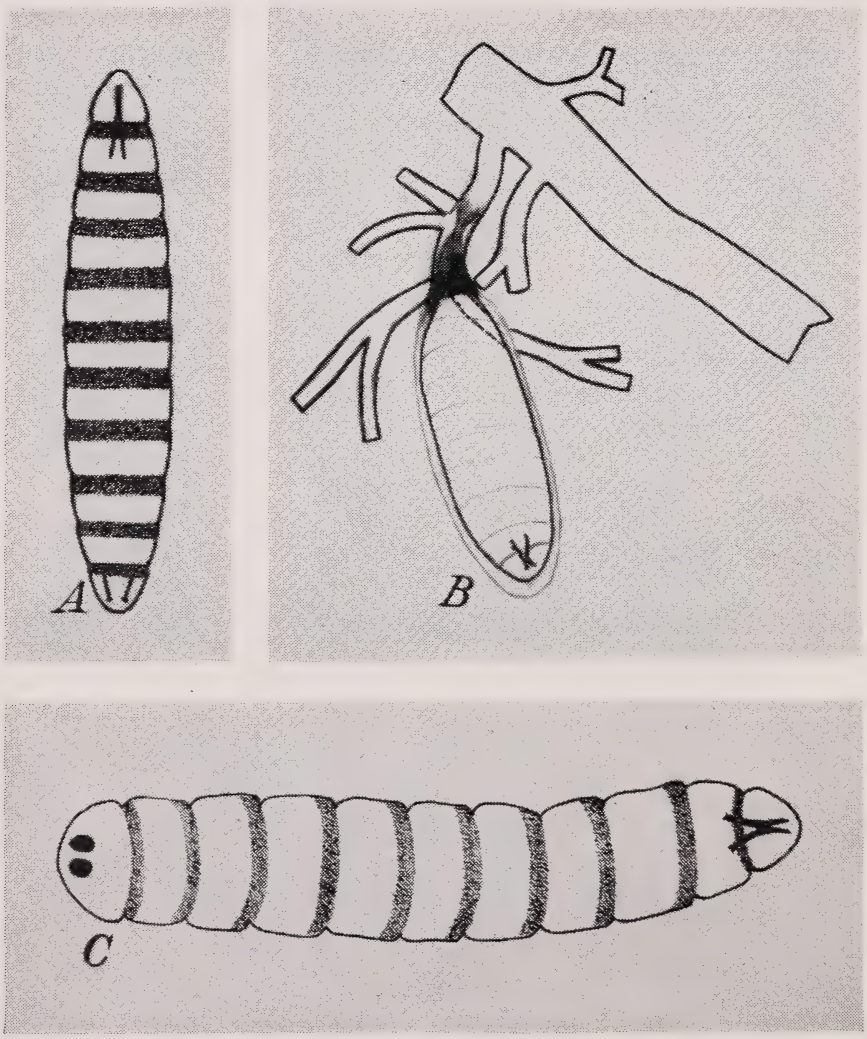

FIGURE 3.—*A*, First-instar larva of *Metagonistylum minense; B*, second-instar larva of *M. minense*, showing attachment to tracheal tubes of the host; *C*, third-instar larva of *M. minense*.

spines. The mandibles are shown in figure 2, *B*. The anal spiracles and the main tracheal trunks are visible.

The second-instar larva measures from 0.75 to 2.25 mm. in length. During the latter half of this instar it is attached at the posterior end by a chitinized funnel to the tracheal trunk of the host as shown in figure 3, *B*. The margins of the segments also bear numerous short spines, but these are not readily visible as in the case of the first- and third-instar larvae.

The third-instar larva (fig. 3, *C*) passes through three distinct phases; during most of this stage it remains attached to the trachea of the host by a chitinized funnel. It then passes a short period free within the body of the host before emergence. Figure 4 shows

FIGURE 4.—Third-instar larva of *Metagonistylum minense* recently emerged from *Diatraea saccharalis* borer. Host borer also contains another parasite larva as yet unemerged.

a third-instar larva just emerged; this host larva also contains a second parasite larva not yet emerged. The size of the third-instar larva varies greatly, depending upon the size of the host and the number of individuals within a single host. However, on the average, a mature third-instar larva at the time of emergence measures from 8 to 10 mm. in length. The body is white with the margins of each segment banded with rows of characteristic black spines. The heavily chitinized anal spiracles are readily visible.

PUPARIUM

The puparium of *Metagonistylum minense* (fig. 5) is oval with the sides nearly parallel and the ends evenly rounded. The length

varies from 5.2 to 9.0 mm. and the width is slightly less than one-third of the length. The puparium when first formed is a light reddish brown but within 48 hours becomes much darker and by the time emergence takes place is dark reddish to blackish brown with distinct finely punctuated bands marking the segments. The puparia of *M. minense* are distinguishable from those of other fly parasites

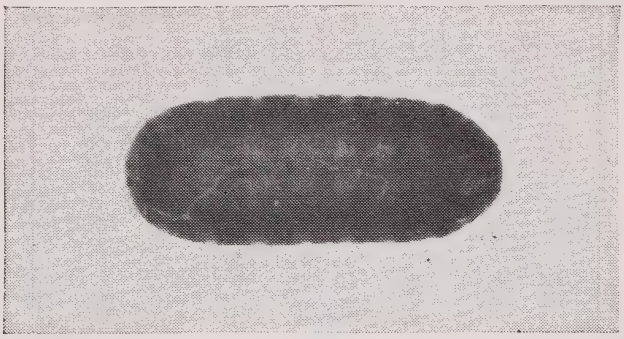

FIGURE 5.—Puparium of *Metagonistylum minense.*

of *Diatraea* by their general shape and the form of the openings of the posterior or anal spiracles, shown in figure 6, *A*.

Jaynes (*11*) has diagrammed the markings found on the puparium of *Theresia claripalpis* (V. d. W.) as shown in figure 6, *B*, and they are quite distinct from those of *M. minense.* In general, the puparia of the two species are similar in appearance and might be confused by inexperienced workers. The puparium of the other common fly

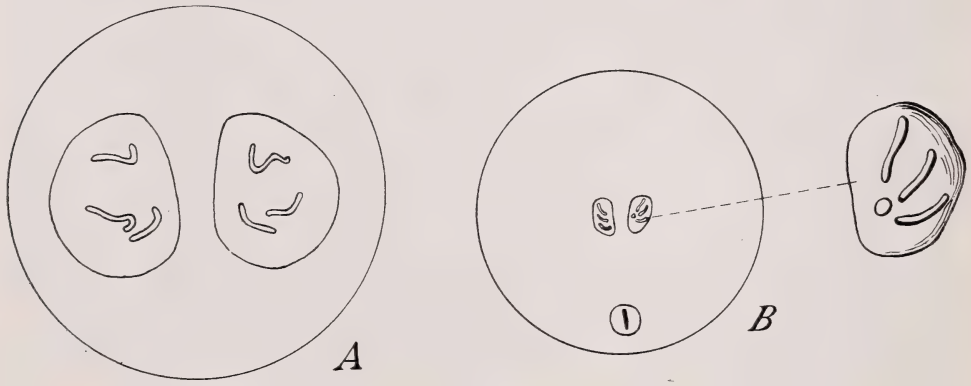

FIGURE 6.—The posterior spiracles of the puparia: *A*, Puparium of *Metagonistylum minense; B*, puparium of *Theresia claripalpis* (After Jaynes).

parasite, *Lixophaga diatraeae* Tns., known to attack *Diatraea*, is much smaller than either of the above and the ventral line of the puparium is slightly concave, which makes it readily distinguishable. The puparium of another fly parasite of little importance, *Leskiomima jaynesi* Ald. is, according to Jaynes (*11*), readily distinguishable by the short spinelike projections at the anterior end, the thoracic spiracle in the form of a projecting tube, and each of the posterior spiracles consists of three protruding spinelike tubes.

BIOLOGY

HABITS OF THE ADULT

The adult of *Metagonistylum minense* is positively phototropic. Under cage conditions in subdued light the flies are inactive and are content to rest on the sides of the cages for long periods of time. When released in the field they appear to be strong fliers and disperse rapidly although on occasion a few may be seen crawling or resting on nearby cane for a considerable period of time after release.

The adults feed readily on sugar water, honey, dry granulated sugar, and sliced sugarcane stalks, and apparently survive equally well on any of these foods.

Under laboratory conditions a few flies have been kept alive as long as 20 days and one fly for 23 days; however, the majority survive for only 12 days or less. A small percentage of the flies die before completion of the gestation period and usually a high mortality results after the ninth day in confinement.

Emergence takes place during a rather definite period. With the Amazon strain under Puerto Rican conditions nearly all of the adults emerge from 10:30 a. m. to 1 p. m., whereas with the São Paulo material the emergence period usually is an hour earlier and of shorter duration. Emergence of both strains has been observed from early morning to late afternoon, but over 95 percent occurs within the above limits.

The proportion of the sexes in laboratory rearings was nearly equal for groups of hundreds of individuals, with the females slightly predominant in the total number counted. However, in some cases, such as counts taken of rearings from a single female, where small numbers of individuals were involved, one sex outnumbered the other as much as 2 to 1.

MATING AND GESTATION

The flies mate readily shortly after emergence. No difficulty has ever been encountered in mating the flies in small glass vials or in various kinds of cloth cages. Cleare (7) has discussed in detail the narrow limits of light intensity necessary to induce mating. While unquestionably the light-intensity factor is important, the flies have been observed to mate under widely varying light conditions. That the majority of matings do take place during a certain light intensity is unquestionably true, and such a light intensity produces a very characteristic flight activity, as stated by Cleare. However, for practical laboratory rearing and colonization, satisfactory mating was obtained by leaving the flies exposed in a place where various light conditions occurred intermittently for a period of 4 to 5 hours after emergence.

Copulation does not always take place at once but may commence with a struggle on the part of the female to free herself or she may accept the attention of the male quietly. After the male gains a position above and slightly to the rear of the female a definite courtship takes place during which time the male taps the female a number of blows with his abdomen. On acceptance, the female raises her abdomen and copulation is effected almost at once.

The copulation period varies considerably, ranging from a few minutes to 20 or 25 minutes, but the average mating lasts about 12

minutes. The same pair may copulate for a second or third time if allowed to remain together. The males mate readily with other females on the same day or some days thereafter. Females have been observed to mate a second day but thereafter generally refuse attention from the males. In a few cases mated females have been found to copulate again on the fourth and even on the fifth day of the gestation period.

The gestation period under Puerto Rican conditions was found to vary slightly with the season. During the warmer periods most females would oviposit to some extent on the fifth day, but during the cooler months the majority were not capable of oviposition until the seventh day. For laboratory purposes the flies were allowed to complete a gestation period of 8 to 10 days in order to insure a maximum number of mature eggs in the ovisac.

OVIPOSITION

A mated female fly that has completed a gestation period of 6 days will oviposit at the slightest stimulus of odor indicating the presence of a *Diatraea* larva. Cleare (*7*) states that there appeared to be no selection on the part of the fly between the stimulus of *D. saccharalis* and *D. canella*, although successful rearings were uncommon with the latter species. Flies have been observed to oviposit on sugarcane, glass plates, or wherever the odor of a *Diatraea* larva was present. When females were retained in the laboratory for periods of 10 or 11 days, oviposition sometimes occurred spontaneously in the cages without apparent stimulation.

When stimulated to oviposition the fly spends a few seconds passing her antennae over and about the stimulus. She then assumes a crouching position, arching the abdomen slightly with the tip pointed down and slightly forward. Oviposition takes place at once and from 1 to 7 eggs are laid at each deposition, 3 or 4 eggs being the usual number. This process may be repeated a number of times before the fly makes any attempt to leave the stimulus. The eggs hatch immediately and only by careful and rapid observation is it possible to detect hatching, although the empty eggshells may be seen.

Under natural conditions the fly normally deposits eggs, but upon dissection of the uterus, either due to mechanical disruption or to exposure of the mature eggs to the air, hatching takes place immediately and the dissected uterus gives the appearance of being filled with larvae. The eggs seldom, if ever, hatch within the uterus. A female 17 days old which had not been given an opportunity or stimulation to oviposit, deposited eggs in a normal manner on the introduction of a *Diatraea* borer.

Unfertilized females, usually after the fourth or fifth day, also react to the stimulus of *Diatraea* and attempt to oviposit, assuming the characteristic raised position of the abdomen and exposing the genitalia, but have not been observed to pass an egg. However, mated females, if exposed to such stimulus before mature eggs are present in the uterus, will not attempt oviposition.

Under field conditions the eggs are undoubtedly laid at the entrance hole made by the *Diatraea* larvae, the frass thrown out by the larval feeding readily serving as a stimulus to induce oviposition.

Jaynes (*11*), in discussing the life history of *Theresia claripalpis*, suggests that the parasite larvae are probably guided to the host by

its odor and by the dark appearance of the burrow entrance. Laboratory observations indicate that *Metagonistylum minense* is more probably guided by a negative phototropic response than by the odor of the host larva. Unquestionably the majority of the parasite larvae perish without even finding a host. The large number of larvae deposited at each oviposition would tend to substantiate such an assumption.

While a single host larva is capable of supporting several parasites, the development of more than 2 or 3 is usually fatal to the host before parasite emergence, and the usual number reared is only 1 or 2. In the laboratory rearing work from 1 to 3 parasite larvae were placed upon a host borer, dependent upon the size of the latter. The greatest number successfully reared from a single host in experimental trials in the laboratory was 5. Clear (*7*) reports 1 isolated instance in the laboratory where 1 host larva was found to contain 7 parasite larvae. Box (*4*) reports the successful rearing of 9 small flies from a single large host borer, laboratory-inoculated with 10 parasite larvae.

LARVAL AND PUPAL STAGES

The penetration of the host by the parasite larva can be observed in the laboratory by using free-crawling host borers. Under such conditions most of the parasite larvae tend to wander aimlessly over the body of the host and, unless supplied with moisture, soon expire. In a few cases an effort to penetrate the host starts immediately. Penetration is effected through the thinly chitinized part of the skin, such as is found between segments. Entry has been observed in nearly every part of the body, but the dorsal side seems to be preferred. The parasite larva rasps the skin of the host rapidly with its mandibles until an entrance is effected and then pushes itself within by contortion of the body segments. The parasite larva usually enters the body in a longitudinal position and at times may be observed for some hours thereafter lying just beneath the skin of the host. If the host borer becomes dry as penetration is proceeding, the parasite larva uses what apparently is the blood oozing from the host to build up a protective coating about itself and thus prevent drying out. In such cases penetration takes much longer, lasting from 1 to 2 hours. If moisture is plentiful penetration is accomplished in from 30 to 40 minutes, the shortest time observed being 21 minutes.

After penetration the first-instar larva remains free in the body cavity for a period of 48 to 72 hours.

During the early stages of the second instar the larva becomes attached to one of the larger tracheal tubes of the host by means of a chitinous funnel. The attachment to the trachea is usually made at or near the extremities of the body of the host larva with the head end of the parasite larva free to feed in the midsection of the host. In cases of multiple parasitism the parasite larvae are usually found lying in a parallel position, attached to the tracheal tubes on opposite sides of the host's body. The length of time spent in the second instar is comparatively short, being observed to be only about 36 to 48 hours. It is difficult to determine the exact limits of the various larval stages, as all such observations are the result of dissection, and the length of one stage in relation to the other can be determined only by using the average for the preceding stage in relation to the condition found at the time of previous dissections.

Third-instar larvae have been found to be present within 84 hours after inoculation, but the majority are not found until 96 to 120 hours after inoculation. The average period spent in the third instar previous to emergence is about 48 hours. Pupation takes place in from 8 to 20 hours after emergence from the host.

The length of the pupal stage averages 9.7 days, but extremes from 7 to 16 days have been noted.

The entire life history from egg to adult will average from 16 to 20 days under semitropical conditions. The temperature fluctuations apparently affect the length of the pupal stage to the greatest extent. While some slowing down or speeding up of the larval stages results from fluctuations in temperature, the variation is so small that it would be extremely difficult to define it as other than within normal limitations.

DEVELOPMENT IN RELATION TO TEMPERATURE

During the course of the laboratory rearing it was found that a few degrees' fluctuation in the mean temperature resulted in variations in the period of development of *Metagonistylum minense*. For the purpose of correlating these variations in the period of development at different temperatures, the developmental period of all those reared individuals which were inoculated and which emerged within the same monthly period was determined and compared with the official monthly temperatures recorded at Mayaguez.

Observations were made on the Amazon strain of *M. minense* from November 1937 to July 1939. During that period the average mean monthly temperature varied from 72° to 80° F., and the period of development varied from 15.3 days to 21.9 days, a fluctuation of 6.6 days.

For the São Paulo strain of *M. minense* observations were made from April 1939 to June 1940, during which time the mean average monthly temperature varied from 74° to 80.1° F. and the period of development from 16.7 to 20.3 days. The temperature fluctuations in relation to development are shown in table 1.

TABLE 1.—*Number of days required under varying temperatures to complete the developmental period of the Amazon and São Paulo strains of Metagonistylum minense*

Temperature fluctuations [1]	Days required for development	
	Amazon strain	São Paulo strain
°F.	*Number*	*Number*
72-75.0	19.3-21.9	20.0-20.3
75-77.0	16.4-18.7	18.6-19.7
77-80.1	15.3-17.3	16.7-17.5

[1] In the case of the São Paulo strain the lowest mean temperature recorded for the observations given was 74° F.

In general a fluctuation of about 1° F. in the mean monthly temperature resulted in slightly less than 1 day difference in the developmental period of the parasite.

As shown in table 1, the São Paulo strain averaged 24 to 48 hours longer than the Amazon strain in the time required for development under similar conditions of temperature.

LABORATORY REARING

The method of artificially rearing *Metagonistylum minense* by the dissection of the uterus to obtain abundant parasite larvae for artificial inoculation was first recognized by Scaramuzza (*16*), being similar to the methods he had used with *Lixophaga diatraeae* Tns. (*15*). Modifications and refinements of the technique for this particular parasite were carefully worked out by Cleare (*7*). Similar methods were used by Dohanian (*8*) and Bartlett [3] in rearing material in British Guiana and Puerto Rico for introduction into Puerto Rico.

In Puerto Rico mated females that have completed at least an 8-day gestation period were found to contain a satisfactory proportion of mature eggs within the uterus. The fly to be used for dissection purposes is lightly anaesthetized and the uterus removed and placed in a small amount of physiological salt solution, 1 gram to 1 liter of distilled water. The narrow end of the uterus is gradually teased open to slowly permit the escape of the parasite larvae. The use of a black plate for such work facilitates the ease with which the larvae may be seen with the naked eye.

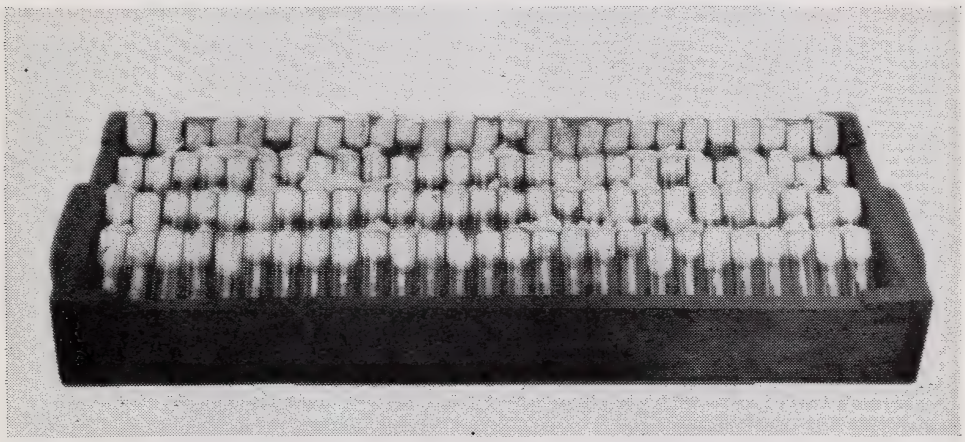

FIGURE 7.—Tray of glass vials containing isolated larvae of *Diatraea saccharalis* parasitized by *Metagonistylum minense*.

The *Diatraea saccharalis* borers to be inoculated are placed in a similar saline solution previous to the transfer of the parasite larva or larvae. This is accomplished by means of a camel-hair brush twisted to a fine point with which the parasite larvae may be lifted without injury.

The handling of the *Diatraea* larvae after inoculation was modified to some extent from the methods used by other workers. The original technique developed by Cleare of placing the larvae in cane shoots was used at first, but later Guatemala grass (*Tripsacum laxum* Nash) was substituted for sugarcane as it was found to be less susceptible to rotting. This change in the host food plant resulted in a change in the method of handling. The sugarcane borers were isolated in glass vials with small pieces of Guatemala grass stems for food. Figure 7 shows a tray for holding such vials. Instead of placing the *Diatraea* borer in a prepared cavity within the shoot, as was

[3] BARTLETT, KENNETH A. THE INTRODUCTION AND COLONIZATION IN PUERTO RICO OF BENEFICIAL INSECTS PARASITIC ON THE SUGARCANE MOTH BORER. Puerto Rico Agr. Expt. Sta. Agr. Notes 78, 8 pp. 1937. [Mimeographed.]

done with cane, the borer was allowed to penetrate the Guatemala grass as a result of normal feeding after inoculation.

When pieces of cane were used as food they were placed in galvanized-iron containers and were not disturbed until emergence of the adult fly took place. In the use of Guatemala grass the pieces were removed from the vials after a few days' isolation and were then placed in galvanized-iron containers to await emergence. It was found advisable to separate the pieces of grass by the use of 1-inch mesh wire netting, two or three pieces being placed in each compartment formed by the mesh of the wire, as shown in figure 8. A ½-inch layer of saw-

FIGURE 8.—Galvanized-iron boxes used for storing Guatemala grass cuttings containing parasitized *Diatraea saccharalis* larvae.

dust was placed on the bottom of the cage to absorb any excess moisture from the rotting grass. Finely powdered crude sulfur was liberally sprinkled over the sawdust, which reduced mite infestation to a minimum.

On emergence from the puparia the flies are collected and placed in cloth cages for mating. Various types of cages were used. A most satisfactory cage is made of an iron framework, 21 inches high, and 15 inches in diameter, with galvanized-iron bottom, and covered with a mosquito netting provided with a sleeve entrance, as shown

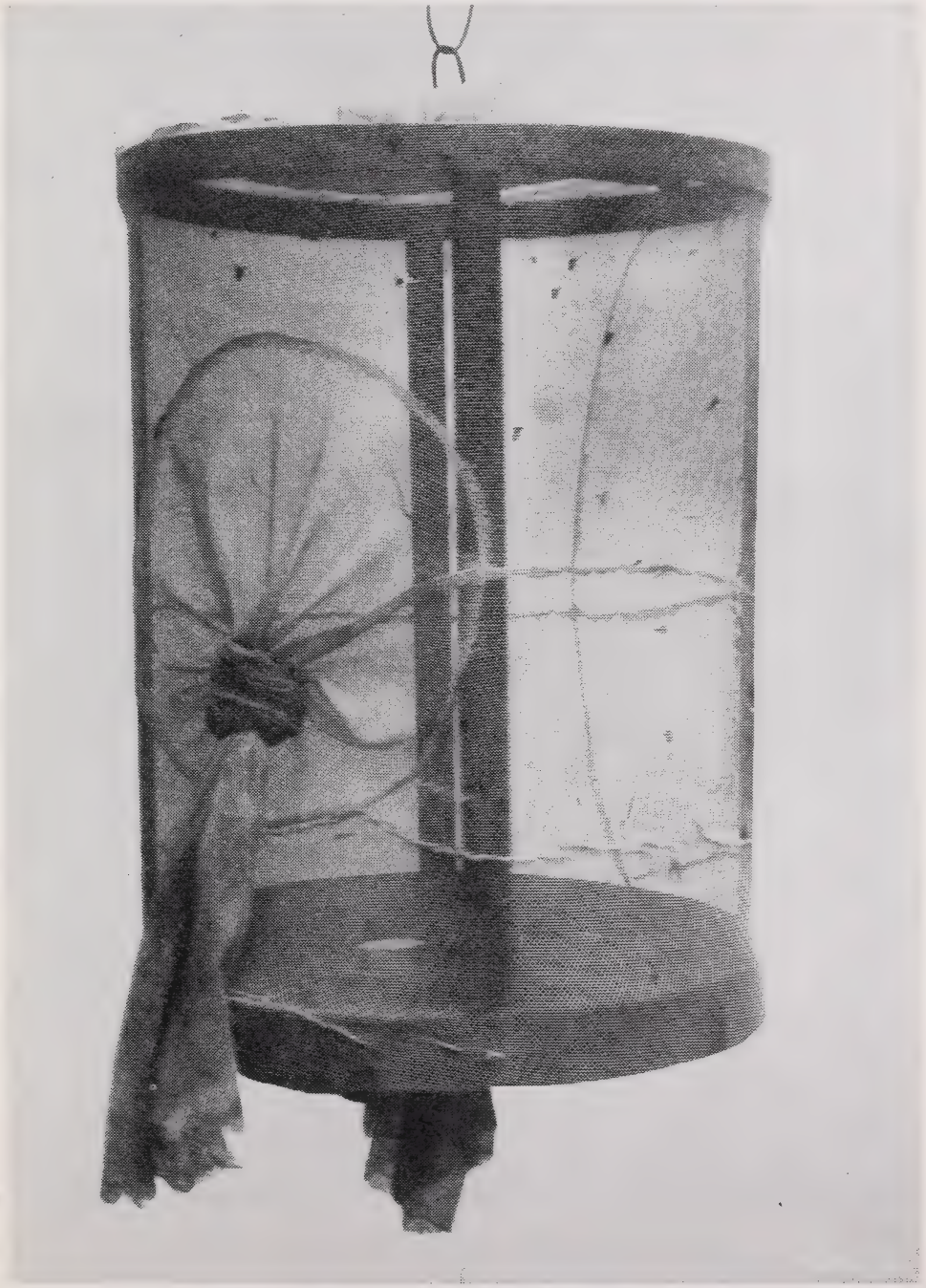

FIGURE 9.—A cage used for mating and storing *Metagonistylum minense* prior to liberation against the sugarcane borer. The cage is 15 inches in diameter by 21 inches long and is covered with coarse bobbinet gathered and tied with a string at top and bottom. At left is shown a sleeve through which the flies are introduced into the cage.

in figure 9. A burlap sack was placed in the bottom to provide moisture; sugar was provided in small glass receptacles. This type of cage was used successfully by Cleare in British Guiana.

HYPERPARASITES

A number of different hyperparasites have been recorded by various writers as attacking the puparia of *Metagonistylum minense*. In its native home in the Amazon, Myers (*14*) reports finding 3 species, a Pteromalid and 2 Encyrtids, but he states that none of these was frequently encountered. In São Paulo, Brazil, Bartlett (*3*) found one species, *Trichopria* sp.[4] At Piracicaba 143 puparia were field collected in sugarcane and 34, or 23.8 percent, were found to be parasitized, and at Campinas 8 of 26 puparia, or 30.8 percent, were parasitized by this species. Cleare (*7*) reports a native species, *Signiphora dipterophaga* Girault, attacking the puparia of *M. minense* in British Guiana, but the percentage destroyed by hyperparasites in all the fields examined was only 3.3 percent. In Puerto Rico no hyperparasites have been encountered attacking the puparia of *M. minense* or of our native dipterous parasite of the sugarcane borer, *Lixophaga diatraeae*.

STATUS IN PUERTO RICO

The original introduction of *Metagonistylum minense* into Puerto Rico was made by the Bureau of Entomology and Plant Quarantine in 1935. The collection and shipment of this material is recorded by Dohanian (*8*) and its colonization in Puerto Rico by Bartlett.[5] Unfortunately this first introduction was made during the dry season and owing apparently to adverse climatic conditions at the time of liberation and for many months thereafter, during which an extended period of drought was experienced, the species failed of establishment.

In October 1936 the biological control program undertaken by the Bureau of Entomology and Plant Quarantine in 1935 was continued as a project of the Puerto Rico Experiment Station of the United States Department of Agriculture. In 1937 a second introduction of this parasite was made possible through the cooperation of the sugar growers of Puerto Rico who financed a trip for the entomologist of the experiment station to British Guiana to obtain further material. The report of this introduction is recorded by Bartlett.[6] In March 1939 the introduction of the São Paulo strain of *M. minense* was made by Bartlett (*3*). An extensive rearing program was initiated in October 1937, through the cooperation of a number of sugar growers of the island who provided *Diatraea saccharalis* borers for rearing *M. minense* in the laboratory. In return for the collection of such material the sugar growers received adult flies for liberation on their properties.

Through July 30, 1940, liberations were made at 19 different points, and a total of 41,177 flies had been liberated. This program is being continued and new points are being colonized as the rearing work per-

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[4] Determination by C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine.
[5] Bartlett, Kenneth A. See footnote 3.
[6] BARTLETT, K. A. THE SECOND INTRODUCTION OF THE AMAZON FLY FROM BRITISH GUIANA INTO PUERTO RICO. Puerto Rico Agr. Expt. Sta. Agr. Notes 86, 4 pp. 1938. [Mimeographed.]

mits, with both biological strains being tested. A liberation of adult flies being made in a sugarcane field is shown in figure 10.

FIGURE 10.—Liberation of adult flies of *Metagonistylum minense* in a sugarcane field.

Initial recoveries of *Metagonistylum minense* were made in the vicinity of many of the liberation points within a few months after colonization was started in 1937.

At the present time the Amazon strain of the fly is considered to be well established in the Añasco Valley region, Mayaguez, the San German Valley region, and on the south coast about Santa Isabel. Recoveries were made at other points but not in sufficient numbers to indicate definite establishment. In general, the percentage of parasitization has been low, but isolated instances have given reason to believe that as the fly spreads throughout the sugarcane-growing areas it will aid materially in the control of *Diatraea saccharalis*. The highest parasitization found had been 46 percent at Mayaguez, 3 years after the original liberation was made. These parasitized borers were collected from infested sweet corn, which is also a favorite host plant of this pest. One collection at Añasco from sugarcane showed 13.8 percent of the borers attacked and at San German 6.9 percent. However, in many collections the parasitization by *M. minense* was 1 percent or less and in many cases it was negative.

SUMMARY

The Amazon fly, *Metagonistylum minense*, a parasite of the sugarcane borer, was first successfully introduced into British Guiana in 1932 and since has been distributed to many of the important sugar-growing areas of the Western Hemisphere. Two physiological strains of the fly are known to occur, one from the Amazon region and one from the southern part of Brazil in the vicinity of São Paulo. The two strains are readily differentiated by the coloring on the abdomen. The Amazon strain has a reddish-yellow cast whereas the São Paulo strain is a melanic and completely black throughout.

The fly is easily propagated in the laboratory by dissection and removal of the uterus to obtain parasite larvae for inoculation purposes. The small semitransparent larvae are placed on *Diatraea* borers and shortly thereafter they enter the body of the host and commence feeding internally. In about 7 days, the full-fed larvae emerge and the host larva is almost completely consumed by the ravenous feeding of the parasites. The puparium formed is dark-brown in color and readily distinguished from other fly parasites of *Diatraea* by the characteristic markings of the anal spiracles. Adult flies emerged in about 10 days, thus completing the life cycle in the short period of from 16 to 20 days.

The Amazon strain of the parasite is considered to be successfully established at a number of different points in Puerto Rico, although to date the percentage of parasitization has been low.

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